

AMENDMENTS TO THE SPECIFICATION

Between the title and the section heading "FIELD OF THE INVENTION," please remove the section heading "BACKGROUND OF THE INVENTION."

After the section entitled "CROSS REFERENCES TO RELATED APPLICATIONS," which was added at page 1 of the specification in the amendment filed on September 19, 2005, please replace the section heading "DESCRIPTION OF PRIOR ART" with the section heading "BACKGROUND OF THE INVENTION."

Please replace the paragraph at page 2, lines 16 through 20 with the following paragraph:

Sequence listing comprising 548,156 genomic sequences, is filed under section 801(a)(i) on electronic medium in computer readable form, attached to the present invention, and is hereby incorporated by reference. Said sequence listing is contained in a self-extracting compressed file named SEQ_LIST.EXE (9,813KB). Compressed file contains 1 file named SEQ_LIST.TXT (82,859KB) the file named "Patent12_Human_PCT_PatentIn_1.txt" (79,122 KB), which was created on June 4, 2008.

Please replace the paragraph at page 7, lines 21-23 with the following paragraph:

Figs. 2, 3, and 4 are schematic diagrams which when taken together provide an analogy that illustrates a conceptual model of the present invention, addressing the genomic differentiation engine. Fig. 2A depicts an analogy for gene expression. Fig. 2B depicts an analogy for differential gene expression between cells.

Please replace the paragraph at page 10, lines 7 and 8 with the following paragraph:

Fig. 23B is a schematic representation of secondary folding of hairpins of the operon-like cluster of Fig. 23A. The hairpins are associated with the following SEQ ID NOs: N2 (SEQ ID NO: 548172); N3 (SEQ ID NO: 548173); MIR23 (SEQ ID NO: 548174); GAM22 (SEQ ID NO: 548175); GAM116 (SEQ ID NO: 548176); N4 (SEQ ID NO: 548177); N6 (SEQ ID NO: 548178); MIR24 (SEQ ID NO: 548179); N7 (SEQ ID NO: 548180);

Please replace the paragraph at page 10, lines 14-17 with the following paragraph:

Fig. 24A is an annotated sequence of EST72223 (SEQ ID NO: 548181) comprising known miRNA gene MIR98 and novel gene GAM25, both detected by the gene detection system of the present invention. The

sequence of EST 72223 includes the four marked sequences: The sequence of the miRNA-98 hairpin in bold (SEQ ID NO: 548182), the sequence of the mature miRNA-98 in bold and underlined (SEQ ID NO: 548183), the sequence of the GAM25 hairpin in bold (SEQ ID NO: 548184) and the sequence of the mature miRNA of GAM25 in bold and underlined (SEQ ID NO: 548185).

Please replace the paragraph at page 22, lines 5-8 with the following paragraph:

Finally, wet lab experiments are preferably conducted in order to validate expression and preferably function of the sample novel genes detected by the BIOINFORMATIC GENE DETECTION ENGINE 100 in the previous step, as designated by reference numeral 132. An example of wet-lab validation of the above mentioned sample novel gene bioinformatically detected by the system is described hereinbelow with reference to Figs. 22A and 22B.

Please replace the paragraphs at page 37, line 30 through page 38, line 24 with the following paragraphs:

First, genes of the novel group of genes of the present invention, referred to here as GAM genes, as designated by reference numeral 146, are located and their function elicited by detecting target proteins they bind and the function of those target proteins, as described hereinabove with reference to Figs. 9 through 15.

Next, genes of a novel group of operon-like genes of the present invention, referred to here as GR genes, as designated by reference numeral 147, are located, by locating clusters of proximally located GAM genes, based on the previous step.

Consequently, the hierarchy of GR and GAM genes is elicited, as designated by reference numeral 148: binding sites for non-protein-binding GAM genes comprised in each GR gene found are sought adjacent to other GR genes. When found, such a binding site indicates that the connection between the GAM and the GR the expression of which it modulates, and thus the hierarchy of the GR genes and the GAM genes they comprise.

Lastly, the function of GR genes and GAM genes which are 'high' in the hierarchy, i.e. GAM genes which modulate expression of other GR genes rather than directly modulating expression of target proteins, may be deduced. A preferred approach is as follows: The function of protein-modulating GAM genes is deducible from the protein which they modulate, provided that the function of these target proteins is known. The function of 'higher' GAM genes may be deduced by comparing the function of protein-modulating GAM genes, as designated by reference numeral 149, with the hierarchical relationships by which the 'higher' GAM genes are connected of the protein-modulating GAM genes. For example, given a group of several protein-modulating GAM genes, which collectively cause a protein expression pattern typical of a certain

cell-type, then a 'higher' GAM gene is sought which modulates expression of GR genes, which perhaps modulate expression of other genes which eventually modulate expression of the given group of protein-modulating GAM genes. The 'higher' GAM gene found in this manner is taken to be responsible for differentiation of that cell-type, as per the conceptual model of the invention described hereinabove with reference to Fig. 6

Please replace the paragraph at page 44, lines 6-26 with the following paragraph:

Fractionation was done by loading up to 500µg per YM100 Amicon Microcon column (Millipore) followed by a 500g centrifugation for 40 minutes at 4°C. Flowthrough "YM100" RNA consisting of about ¼ of the total RNA was used for library preparation or fractionated further by loading onto a YM30 Amicon Microcon column (Millipore) followed by a 13,500g centrifugation for 25 minutes at 4°C. Flowthrough "YM30" was used for library preparation as is and consists of less than 0.5% of total RNA. For the both the "ligation" and the "One-tailed" libraries RNA was dephosphorylated and ligated to an RNA (lowercase)-DNA (UPPERCASE) hybrid 5'-phosphorylated, 3' idT blocked 3'-adapter (5'-P-uuuAACCGCATTCTC-idT-3' Dharmacon # P-002045-01-05) (SEQ ID NO: 548157) (as elaborated in Elbashir et al 2001) resulting in ligation only of RNase III type cleavage products. 3'-Ligated RNA was excised and purified from a half 6%, half 13% polyacrylamide gel to remove excess adapter with a Nanosep 0.2µM centrifugal device (Pall) according to instructions, and precipitated with glycogen and 3 volumes of Ethanol. Pellet was resuspended in a minimal volume of water. For the "ligation" library a DNA (UPPERCASE)-RNA (lowercase) hybrid 5'-adapter (5'-TACTAATACGACTCACTaaa-3' Dharmacon # P-002046-01-05) (SEQ ID NO: 548158) was ligated to the 3'-adapted RNA, reverse transcribed with "EcoRI-RT" : (5'-GACTAGCTGGAATTCAAGGATGCGGTAAA-3') (SEQ ID NO: 548159), PCR amplified with two external primers essentially as in Elbashir et al 2001 except that primers were "EcoRI-RT" and "PstI Fwd" (5'-CAGCCAACGCTGCAGATACGACTCACTAAA-3') (SEQ ID NO: 548160). This PCR product was used as a template for a second round of PCR with one hemispecific and one external primer or with two hemispecific primers.

Please replace the paragraph at page 44, line 27 through page 45, line 11 and the paragraph at page 45, lines 12-19, with the following two paragraphs:

For the "One tailed" library the 3'-Adapted RNA was annealed to 20pmol primer "EcRI RT" by heating to 70°C and cooling 0.1°C/sec to 30°C and then reverse transcribed with Superscript II RT (According to instructions, Invitrogen) in a 20µl volume for 10 alternating 5 minute cycles of 37°C and 45°C. Subsequently, RNA was digested with 1µl 2M NaOH, 2mM EDTA at 65°C for 10 minutes. cDNA was loaded on a polyacrylamide gel, excised and gel-purified from excess primer as

above (invisible, judged by primer run alongside) and resuspended in 13µl of water. Purified cDNA was then oligo-dC tailed with 400U of recombinant terminal transferase (Roche molecular biochemicals), 1µl 100µM dCTP, 1µl 15mM CoCl₂, and 4µl reaction buffer, to a final volume of 20µl for 15 minutes at 37°C. Reaction was stopped with 2µl 0.2M EDTA and 15µl 3M NaOAc pH 5.2. Volume was adjusted to 150µl with water, Phenol : Bromochloropropane 10:1 extracted and subsequently precipitated with glycogen and 3 volumes of Ethanol. C-tailed cDNA was used as a template for PCR with the external primers "T3-PstBsg(G/I)₁₈" (5'-AATTAACCCTCACTAAAGGCTGCAGGTGC AGGIGGGIIGGGIIGGGIIGN-3' [SEQ ID NO: 548161] where I stands for Inosine and N for any of the 4 possible deoxynucleotides), and with "EcoRI Nested" (5'-GGAATTCAAGGATGCGGTTA-3') (SEQ ID NO: 548162). This PCR product was used as a template for a second round of PCR with one hemispecific and one external primer or with two hemispecific primers.

Hemispecific primers were constructed for each predicted GAM by an in-house program designed to choose about half of the 5' or 3' sequence of the GAM corresponding to a TM° of about 30°-34°C constrained by an optimized 3' clamp, appended to the cloning adapter sequence (for "One-tailed" libraries 5'-GGNNGGGNNG (SEQ ID NO: 548163) on the 5' end of the GAM, or TTTAACCGCATC-3' (SEQ ID NO: 548164) on the 3' end of the GAM. For "Ligation" libraries the same 3' adapter and 5'-CGACTCACTAAA (SEQ ID NO: 548165) on the 5' end). Consequently, a fully complementary primer of a TM° higher than 60°C was created covering only one half of the GAM sequence permitting the unbiased elucidation by sequencing of the other half.

Please replace the paragraphs at page 49, line 20 through page 50, line 4, with the following paragraphs:

Transcript products were 705nt (EST72223), 102nt (MIR98), 125nt (GAM25) long. EST72223 was PCR amplified with T7-EST 72223 forward primer:

5'-TAATACGACTCACTATAGGCCCTTATTAGAGGATTCTGCT-3'
(SEQ ID NO: 548166)

and T3-EST72223 reverse primer:

5'-AATTAACCCTCACTAAAGGTTTTTTTTTCCTGAGACAGAGT-3'
(SEQ ID NO: 548167).

MIR98 was PCR amplified using EST72223 as a template with T7MIR98 forward primer:

5'-TAATACGACTCACTATAGGGTGAGGTAGTAAGTTGTATTGTT-3' (SEQ ID NO: 548168)

and T3MIR98 reverse primer:

5'-
AATTAACCCTCACTAAAGGGAAAGTAGTAAGTTGTATAGTT-3'
(SEQ ID NO: 548169).

GAM25 was PCR amplified using EST72223 as a template with GAM25 forward primer: 5'-GAGGCAGGAGAATTGCTTGA- 3' (SEQ ID NO: 548170) and T3-EST72223 reverse primer:

5'-AATTAACCCTCACTAAAGGCCTGAGACAGAGTCTTGCTC-3'
(SEQ ID NO: 548171).